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RESEARCH ARTICLE

Antibiotic Susceptibility Profiles of Bacteria from Diabetic Foot Infections in Selected Teaching Hospitals in Southwestern Nigeria

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ABSTRACT

One of the serious complications associated with diabetes is foot ulcer and this condition affects the quality of life in patients in all classes, races and ages. Chronic wounds are prone to colonization by wide array of microorganisms which could be extremely hazardous to patients if effective and timely therapeutic intervention is not made. This study was conducted to determine the antibiotic susceptibility profiles of bacteria from diabetic foot infections in southwestern Nigeria. Samples were collected from a total of 142 diabetic foot ulcer patients with moistened sterile cotton swabs. Nutrient agar, Mac-Conkey agar, blood agar and mannitol salt agar media were used for the isolation of total viable bacteria, Gram-negative non-spore forming lactose fermenters, fastidious bacteria and staphylococci, respectively. Morphological and biochemical characteristics of pure cultures were determined in accordance with standard laboratory criteria. API 20 E and API 20 NE were used for the confirmation of identity of the bacterial isolates. The disc diffusion technique was employed for the determination of antibiotic susceptibility of bacterial isolates in accordance with standard procedures. The antibiotics investigated included amikacin, amoxicillin, ampicillin, ceftazidime, cefazolin, ceftriaxone, chloramphenicol, ciprofloxacin, clindamycin, gentamicin, imipenem, linezolid, methicillin, netilmicin, ofloxacin, oxacillin, penicillin, piperacilin, sulfamethoxazole, trimethoprim and vancomycin. One hundred and seventy-seven isolates were encountered and these were characterized into eleven bacterial species. These included *Staphylococcus aureus* (22.03%), *Pseudomonas aeruginosa* (16.95%), *Staphylococcus epidermidis* (12.43%), *Proteus mirabilis* (8.48%), *Klebsiella pneumoniae* (7.91%), *E. coli* (7.35%), *Staphylococcus saprophyticus* (6.78%), *Streptococcus pyogenes* (5.65%), *Morganella morganii* (5.09%), *Citrobacter freundii* (4.52%) and *Acinetobacter baumannii* (2.83%). Gram-negative bacteria showed 76.99% susceptibility to the antibiotics while 22.85% was resistant. Gram-positive bacteria showed 93.75% susceptibility and 5.01% resistance to the antibiotics. This study revealed that there is no definite aetiologic bacterial agent for diabetic foot infections and many of the associated bacteria are sensitive to certain antibiotics.

Keywords: Antibiotics, bacteria, diabetes, foot ulcer, infections, resistance, susceptibility.

1 Introduction

Diabetes mellitus represents a major public health threat worldwide with an estimated prevalence in 2014 of 422 million patients [1]. A serious complication of diabetes is the development of foot ulcers. World Health Organization (WHO) considered diabetes as one of the twentieth-century epidemics and the most prevalent endocrine disease worldwide with about 10% of global adult population standing the risk of being affected [2]. This disease condition is associated with severe secondary and highly impairing pathologies which include vascular disease with high risk for heart failure and stroke, kidney failure requiring dialysis or transplantation, and blindness. The most devastating conditions in these patients include chronic wounds caused by an impaired immune response and an associated high microbial burden that frequently leads to amputation, mainly of the lower limbs [2], [3]. Every individual is prone to securing skin lesions during a lifetime and which usually heal up without any special care or treatment, but a simple tiny scratch could become a terrible condition for diabetic patients. The lesion could graduate into seriously infected chronic wound leading to amputation or even death [4], [5]. A contributing factor usually is skin microbiota of diabetic patients [6] - [10].

Naturally healing of diabetic chronic wounds is difficult and this makes some degree of therapeutic intervention imperative [11]. Corresponding clinical treatments are usually initiated after diagnosis of the wound chronic stage. Compromised tissues debridement, use of specialized dressings, and appropriate use of antibiotics are the most frequently used approaches to combating diabetic chronic wounds [12]. Success rates are, however, still not satisfactory as no significant improvement has been reported in more than fifty-percent of the patients to this approach. Hence, hospitalization and amputation were often the resulting outcome [2], [13], [14]. Inappropriate antibiotic administration could further impair wound healing progression in many patients [15], [16], and as physicians were left with no options, persistent administration systemic antibiotics becomes the order of the day [12], with the hope that something changes in the health condition of the patient that allows the wound to enter a remission stage [11]. There is no doubt that this is a life-threatening problem which requires urgent chemotherapeutic interventions, hence the need to relentlessly determine the sensitivity of associated microorganisms to conventional antibiotics. Based on the severity and risk associated with foot ulcers, and variations of reports among different geographical places and periods, it becomes imperative to characterize and determine the antibiotic susceptibility patterns of the bacteria from diabetic foot infections in southwestern Nigeria.

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2 Materials and Methods

2.1 Collection of Samples

Ethical clearances were obtained from the institutional ethical committees before collection of samples. Samples (pus, wound exudates) collections were undertaken in medical wards between October, 2016 and March, 2017. Wounds were washed vigorously with normal saline solution and discharges from margins and edges of ulcer were collected from a total of 142 patients (105 in-patients and 37 out-patients). Sixty-seven (67) samples were obtained from University College Hospital (UCH), Ibadan while 75 samples were obtained from Olabisi Onabanjo University Teaching Hospital (OOUTH), Sagamu. Samples were collected with sterile cotton swabs already moistened with sterile saline to prevent drying. The swabs were transported to the laboratory in sterile containers immediately after collection. Samples which were not analyzed within four hours of collection were discarded.

2.2 Microbiological Analysis

Swabs were separately inoculated on appropriate agar media for cultivation and enumeration of associated bacteria. Nutrient agar (Oxoid, England), Mac-Conkey agar (Oxoid, England), blood agar (Oxoid, England) and mannitol salt agar (Oxoid, England) media were used for the isolation of total viable bacteria, Gram-negative non-spore forming lactose fermenters, fastidious bacteria and staphylococci, respectively. The inoculated plates were incubated at 37^{o}C for 24 hours. Plates with mixed cultures were sub-cultured to obtain pure colonies of bacteria. Morphological and biochemical characteristics of the discreet colonies were determined in accordance with standard laboratory criteria. The tests performed include Gram staining, motility, catalase, oxidase, indole, methyl-red, VogesProskauer, urease, citrate utilization, starch hydrolysis, nitrate reduction and sugar fermentation test using glucose, sucrose, arabinose, maltose, xylose, galactose, sorbitol, invositol, raffinose and frauction while API 20 E and API 20 NE were used for the confirmation of identity of the bacterial isolates.

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2.3 Antibiotic Susceptibility Test

2.3.1 Standardization of Inoculum

Cultures of bacteria were cultivated on nutrient agar (Oxoid, England) plates and plates incubated for 24 hours at 37°C. About 100 µl of bacterial cells was dispensed in sterile normal saline to obtain the turbidity of 0.5 McFarland standard, which is a solution of barium sulphate prepared from 0.6 ml of 1% barium chloride added to 99.4 ml of sulphuric acid [17], [18].

2.3.2 Sensitivity Assay

The disc diffusion technique was employed for the determination of antibiotic susceptibility of bacterial isolates in accordance with standard procedures. Young actively growing cultures of bacteria were obtained on nutrient agar (Oxoid, England) plates by overnight incubation at 37°C. Muller Hinton agar (Oxoid, England) media was prepared in sterile Petri dishes. Sterilized swabs were dipped in overnight cultures and spread evenly over the media. The various antibiotic discs were aseptically placed over the media and incubated overnight at 37°C. The conventional antibiotics investigated in this study included amikacin (30 µg), amoxicillin (30 µg), ampicillin (10 µg), ceftazidime (30 µg), cefazolin (30 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg),clindamycin (2 µg), gentamicin (10 µg), imipenem (10 µg), linezolid (30 µg), methicillin (5 µg), netilmicin (30 µg), ofloxacin (5 µg), oxacillin (2 µg), penicillin (10 µg), piperacilin (100 µg), sulfamethoxazole (25 µg), trimethoprim (5 µg) and vancomycin (30 µg) [18].

3 Results and Discussion

A total of 142 samples, comprising of 105 in-patients and 37 out-patients, were collected. Out of the total samples, 75 (56 in-patients and 19 out-patients) were collected from UCH, Ibadan while 67 (49 in-patients and 18 out-patients) were obtained from OOUTH, Sagamu (Table 1). Table 2 showed the occurrence of bacteria among diabetic foot ulcer in- and out-patients visiting teaching hospitals in southwestern Nigeria. The organisms were distributed between the in-patients and out-patients visiting the teaching hospitals. Invariably, both the in-patients and out-patients contributed to the bacterial diversity encountered in this study. Table 3 showed the morphological and biochemical characteristics of bacteria isolated from diabetic foot ulcer in selected visiting hospitals in southwestern Nigeria. A total of one hundred and seventy-seven (177) isolates were encountered in this study and these were characterized as eleven (11) bacterial species. They were *Acinetobacter baumannii*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus* and *Streptococcus pyogenes*.

Table 1: *Details of sample collection from patients from selected teaching hospitals in southwestern Nigeria*

Sampling Site	In-patient	Out-patient	Total Number of Samples
UCH, Ibadan	56	19	75
OOUTH, Sagamu	49	18	67
Total	105	37	142

Table 2: *Occurrence of bacteria among diabetic foot ulcer in- and out-patients visiting teaching hospitals in southwestern Nigeria*

Bacteria	**In-patient**	**Out-patient**	**Total number of bacteria**
Acinetobacter baumannii	4	1	5
Citrobacter freundii	5	3	8
Escherichia coli	9	4	13
Klebsiella pneumonia	9	5	14
Morganella morganii	7	2	9
Proteus mirabilis	11	4	15
Pseudomonas aeruginosa	23	7	30
Staphylococcus aureus	30	9	39
Staphylococcus epidermidis	17	5	22
Staphylococcus saprophyticus	9	3	12
Streptococcus pyogenes	6	4	10
Total	**130**	**47**	**177**

Table 3: *Morphological and biochemical characteristics of bacteria isolated from diabetic foot ulcer patients visiting teaching hospitals in southwestern Nigeria*

Gram Reaction	Cellular morphology	Catalase	Oxidase	Indole	Motility	Methyl-Red	Voges Proskauer	Urease activity	Citrate Utilization	Starch Hydrolysis	Gelatin Hydrolysis	Casein Hydrolysis	Spore test	NO_3 Reduction	Glucose	Sucrose	Arabinose	Maltose	Mannitol	Xylose	Galactose	Sorbitol	Inositol	Raffinose	Frauction	Number of isolates showing characteristics	Most Probable Identity
-ve	Cb	+	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	5	*Acinetobacter baumannii*
+ve	R	+	+	+	+	+	-	-	+	-	-	-	-	-	+	+	-	+	+	-	-	+	-	+	+	8	*Citrobacter freundii*
-ve	R	-	+	-	+	+	+	-	+	-	-	+	+	+	+	+	+	-	+	+	-	-	-	-	+	13	*Escherichia coli*
-ve	R	+	-	-	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	-	+	14	*K. pneumoniae*
-ve	R	+	-	+	+	+	-	+	-	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	9	*Morganella morganii*
-ve	R	+	+	-	+	-	+	+	+	-	+	-	-	+	+	+	+	+	+	+	+	-	-	+	+	15	*P. aeruginosa*
-ve	R	+	-	-	+	+	-	+	+	+	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	30	*Proteus mirabilis*
+ve	C	+	-	-	-	-	+	+	-	-	+	+	-	+	+	+	-	+	+	-	+	-	-	-	+	39	*S. aureus*
+ve	C	+	-	-	-	-	+	+	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	+	22	*S. epidermidis*
+ve	C	+	-	-	-	-	+	+	-	-	+	+	-	+	+	+	-	+	+	-	+	-	-	-	+	12	*S. saprophyticus*
+ve	C	-	-	-	-	+	+	+	+	-	+	+	+	-	+	+	-	+	-	-	+	-	-	-	-	10	*Streptococcus pyogenes*

Keys: Cb = Coccobacilli; R = Rods; C = Cocci; + = Positive reaction; - = Negative reaction; ND = Not determined

The percentage occurrences of bacteria isolated from diabetic foot ulcer in-patients and out-patients were shown in Figure 1. *Staphylococcus aureus* had the highest percentage occurrence of 23.08% and 19.15% in in-patients and out-patients, respectively. This was followed by *P. aeruginosa* with 17.69% (in-patient) and 14.89% (out-patients). Among the in-patients, *S. epidermidis* had the next percentage occurrence of 13.08%, followed by Proteus mirabilis (8.46%), *E. coli* (6.92%), *S. saprophyticus* (6.92%), *Morganella morganii* (5.39%), *Streptococcus pyogenes* (4.62%), *C. freundii* (3.85%) while *Acinetobacter baumannii* had the lowest occurrence of 3.08%. However, among the out-patients, *Acinetobacter baumannii* with percentage occurrence of 12.13% was next to *P. aeruginosa* (14.89%) while *K. pneumoniae*, *S. epidermidis*, *E. coli, S. pyogenes*, *C. freundii* and *M. morganii* had percentage occurrences of 10.64%, 10.64%, 8.51%, 8.51%, 6.38% and 4.25%, respectively.

The overall percentage occurrences of the bacterial species associated with diabetic foot ulcers was shown in Figure 2. *S. aureus* had the highest occurrence of 22.03%, followed by *P. aeruginosa* (16.95%), *S. epidermidis* (12.43%), *P. mirabilis* (8.48%), *K. pneumoniae* (7.91%), *E. coli* (7.35%), *S. saprophyticus* (6.78%), *S. pyogenes* (5.65%), *M. morganii* (5.09%), *C. freundii* (4.52%) while *A. baumannii* (2.83%) had the lowest occurrence. Percentage occurrence of Gram-positive and Gram-negative bacteria in diabetic foot ulcers was given in Figure 3. Results showed that there were a greater number of Gram-negative bacteria than Gram-positive bacteria associated with diabetic foot ulcers investigated in this study. The percentage occurrence of Gram-negative bacteria was 53% while that of Gram-positive bacteria was 47%.

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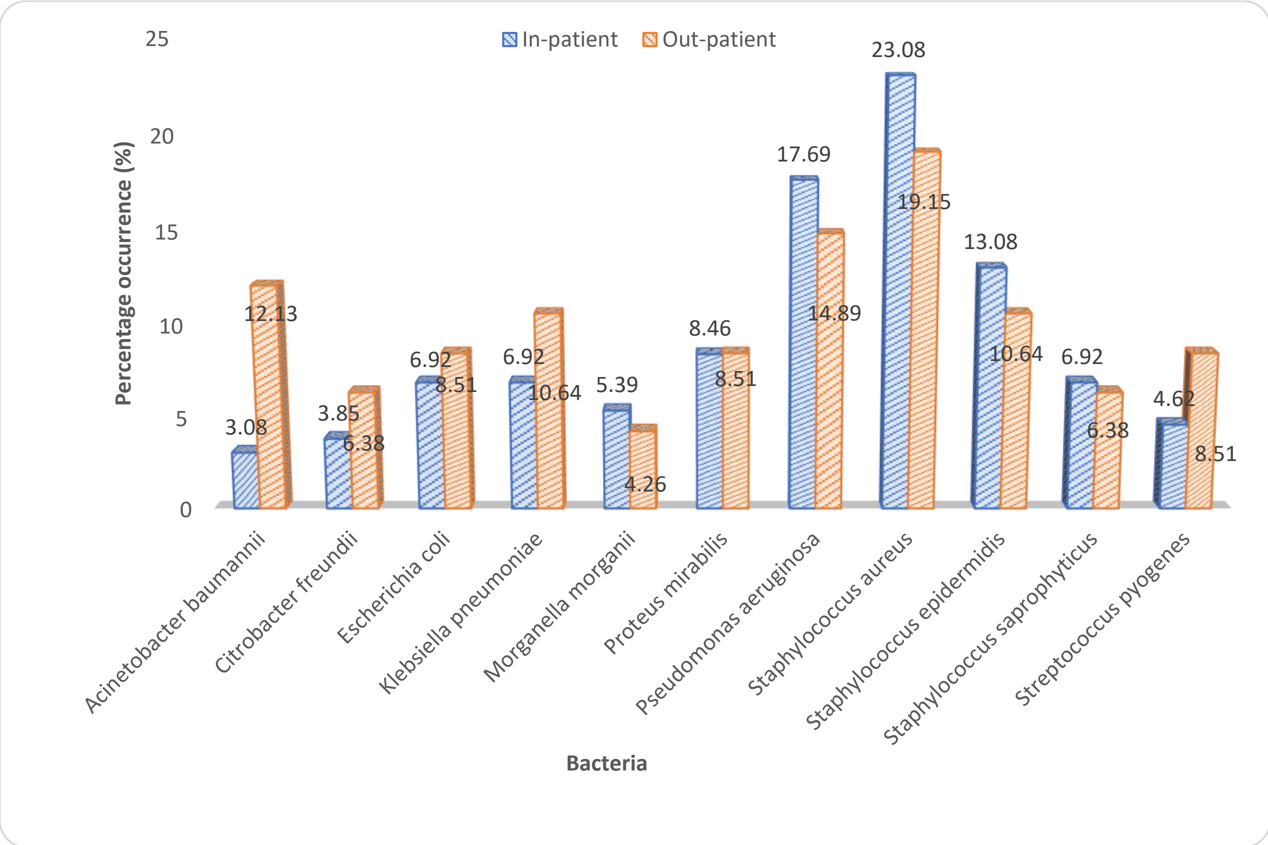

Figure 1: *Percentage occurrence of bacteria isolated from diabetic foot ulcer in-patients and out-patients visiting teaching hospitals in southwestern Nigeria*

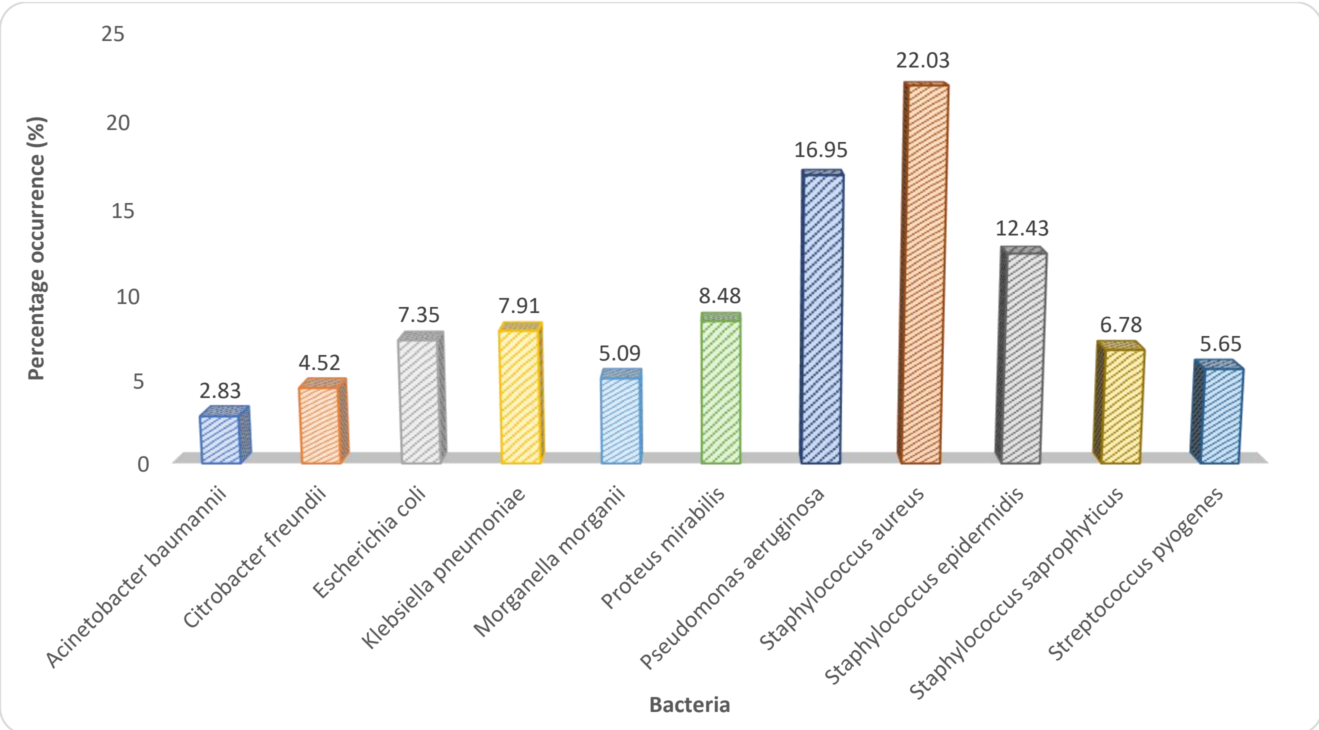

Figure 2: *Overall percentage occurrence of bacteria associated with diabetic foot ulcer from patients visiting teaching hospitals in southwestern Nigeria*

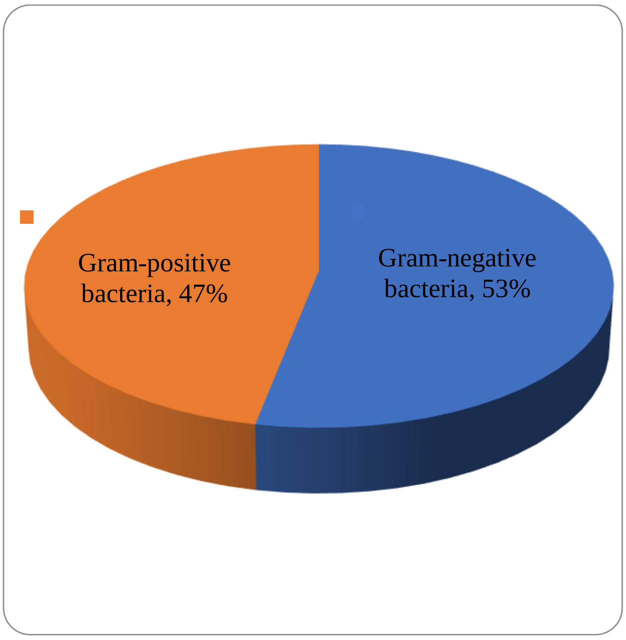

Figure 3: *Percentage occurrence of Gram-positive and Gram-negative bacteria in diabetic foot ulcers from selected visiting hospitals in southwestern Nigeria*

Tables 4a and 4b showed the antibiotic susceptibility and resistance profiles of Gram-negative bacteria isolated from diabetic foot ulcers. Generally, most strains of Gram-negative bacterial species encountered in this study were susceptible to many of the antibiotics investigated. *Acinetobacter baumannii* showed 100% susceptibility to amikacin, amoxicillin, ampicillin, ceftazidime, ceftriaxone, clindamycin, imipenem, methicillin and piperacilin 40%, 20%, 60% and 60% resistances were shown to chloramphenicol, gentamycin, netilmicin and ofloxacin, respectively. *C. freundii* showed 100% sensitivity to amikacin, ceftazidime, cefazolin, ceftriaxone, chloramphenicol, clindamycin, gentamycin, imipenem, methicillin, ofloxacin and piperacillin while 62.5%, 37.5% and 37.5% resistances were shown to amoxicillin, ampicillin and netilmicin, respectively. *E. coli* strains showed 100%, 84.6%, 84.6%, 15.4% and 15.4% resistances to amoxicillin, ampicillin, cefazolin, methicillin and netilmicin, respectively while 100% susceptibility was shown to other antibiotics except amikacin to which 92.3% sensitivity and 7.7% intermediate sensitivity were shown. All strains of *K. pneumoniae* isolated were sensitive to amikacin, ceftazidime, ceftriaxone, clindamycin, gentamycin, imipenem, ofloxacin and piperacillin while all strains were resistant to amoxicillin and ampicillin. However, 64.3%, 21.4%, 28.6% and 28.6% of strains exhibited resistance to cefazolin, chloramphenicol, methicillin and netilmicin, respectively. All *M. morganii* strains were sensitive to all antibiotics except amoxicillin and ampicillin to which 22.2% and 44.4%, resistances were shown respectively.

P. mirabilis exhibited 100% susceptibility to amikacin, ceftazidime, ceftriaxone, clindamycin, gentamycin, imipenem, methicillin, netilmicin, ofloxacin and piperacillin while strains showed 100% resistance to amoxicillin and ampicillin. However, the strains of organism exhibited 26.7%, 13.3% and 20% to cefazolin, chloramphenicol and nitilmicin, respectively. *P. aeruginosa* strains showed 100% resistance to amoxicillin and ampicillin; 13.3%, 63.3%, 60%, 23.3%, 30%, 23.3% and 73.3% resistances were exhibited to amikacin, cefazolin, chloramphenicol, clindamycin, gentamycin, methicillin and netilmicin, respectively.

The antibiotic susceptibility and resistance profiles of Gram-positive bacteria isolated from diabetic foot ulcers were shown in Tables 5a and 5b. All *S. aureus* strains were sensitive to amikacin, amoxicillin, ampicillin, ceftriaxone, imipenem, linezolid, netilmicin, ofloxacin, penicillin, piperacillin, sulphamethazole and trimethoprim; strains showed 71.8% sensitivity and 28.2% intermediate sensitivity to vancomycin while 12.8%, 20.5%, 28.2% and 15.5% resistances were exhibited to ceftazidime, cefazolin, ciprofloxacin and gentamycin, respectively.

S. epidermidis strains showed 27.3%, 9.1%, 18.2% and 59.1% resistances to ampicillin, ceftazidime, penicillin and vancomycin, respectively while strains exhibited 100% sensitivity to other antibiotics. Strains of *S. saprophyticus* exhibited 25%, 16.7% and 66.7% resistances to penicillin, piperacillin and vancomycin, respectively; strains exhibited intermediate susceptibilities of 8.3%, 16.7% and 16.7% to ceftazidime, gentamycin and vancomycin, respectively while 100% susceptibility was shown to the remaining antibiotics. Strains of *S. pyogenes* were sensitive to all antibiotics except ampicillin, netilmicin and penicillin to which 30%, 10% and 30% resistances were shown, respectively while 20% of strains showed intermediate susceptibility to vancomycin.

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Table 4a: *Antibiotic susceptibility and resistance profiles of Gram-negative bacteria isolated from diabetic foot ulcer patients visiting selected visiting hospitals in southwestern Nigeria*

Bacteria		Amikacin	Amoxicillin	Ampicillin	Ceftazidime	Cefazolin	Ceftriaxone	Chloram-phenicol
Acinetobacter baumannii (n = 5)	S	5 100%	5 100%	5 100%	5 100%	5 100%	5 100%	3 60%
	I	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	2 40%
Citrobacter freundii (n = 8)	S	8 100%	3 37.5%	5 62.5%	8 100%	8 100%	8 100%	8 100%
	I	0	0	0	0	0	0	0
	R	0	5 62.5%	3 37.5%	0	0	0	0
Escherichia coli (n = 13)	S	12 92.3%	0	2 15.4%	13 100%	2 15.4%	13 100%	13 100%
	I	1 7.7%	0	0	0	0	0	0
	R	0	13 100%	11 84.6%	0	11 84.6%	0	0
Klebsiella pneumoniae (n = 14)	S	14 100%	0	0	14 100%	5 35.7%	14 100%	11 78.6%
	I	0	0	0	0	0	0	0
	R	0	14 100%	14 100%	0	9 64.3%	0	3 21.4%
Morganella morganii (n = 9)	S	9 100%	7 77.8	5 55.6%	9 100%	9 100%	9 100%	9 100%
	I	0	0	0	0	0	0	0
	R	0	2 22.2%	4 44.4%	0	0	0	0
Proteus mirabilis (n = 15)	S	15 100%	0	0	15 100%	11 73.3%	15 100%	13 86.7%
	I	0	0	0	0	0	0	0
	R	0	15 100%	15 100%	0	4 26.7%	0	2 13.3%
Pseudomonas aeruginosa (n = 30)	S	25 83.3%	0	0	30 100%	11 36.7%	30 100%	12 40%
	I	1 3.3%	0	0	0	0	0	0
	R	4 13.3%	30 100%	30 100%	0	19 63.3%	0	18 60%

Keys: S = Susceptible or Sensitive; I = Intermediately Susceptible/Sensitive and R = Resistant

Table 4b: *Antibiotic susceptibility and resistance profiles of Gram-negative bacteria isolated from diabetic foot ulcer patients visiting selected visiting hospitals in southwestern Nigeria*

Bacteria		Clindamycin	Gentamycin	Imipenem	Methicillin	Netilmicin	Ofloxacin	Piperacilin
Acinetobacter baumannii (n = 5)	S	5 100%	4 80%	5 100%	5 100%	2 40%	2 40%	5 100%
	I	0	0	0	0	0	0	0
	R	0	1 20%	0	0	3 60%	3 60%	0
Citrobacter freundii (n = 8)	S	8 100%	8 100%	8 100%	8 100%	5 62.5%	8 100%	8 100%
	I	0	0	0	0	0	0	0
	R	0	0	0	0	3 37.5%	0	0
Escherichia coli (n = 13)	S	13 100%	13 100%	13 100%	11 84.6%	11 84.6%	13 100%	13 100%
	I	0	0	0	0	0	0	0
	R	0	0	0	2 15.4%	2 15.4%	0	0
Klebsiella pneumoniae (n = 14)	S	14 100%	14 100%	14 100%	10 71.4%	10 71.4%	14 100%	14 100%
	I	0	0	0	0	0	0	0
	R	0	0	0	4 28.6%	4 28.6%	0	0
Morganella morganii (n = 9)	S	9 100%	9 100%	9 100%	9 100%	9 100%	9 100%	9 100%
	I	0	0	0	0	0	0	0
	R	0	0	0	0	0	0	0
Proteus mirabilis (n = 15)	S	15 100%	15 100%	15 100%	15 100%	12 80%	15 100%	15 100%
	I	0	0	0	0	0	0	0
	R	0	0	0	0	3 20%	0	0
Pseudomonas aeruginosa (n = 30)	S	23 76.7%	21 70%	30 100%	23 76.7%	8 26.7%	30 100%	30 100%
	I	0	0	0	0	0	0	0
	R	7 23.3%	9 30%	0	7 23.3%	22 73.3%	0	0

Keys: S = Susceptible or Sensitive; I = Intermediately Susceptible/Sensitive and R = Resistant

Table 5a: *Antibiotic susceptibility and resistance profiles of Gram-positive bacteria isolated from diabetic foot ulcer patients visiting selected visiting hospitals in southwestern Nigeria*

Bacteria		Ami-kacin	Amoxi-cillin	Ampi-cillin	Cefta-zidime	Cefa-zolin	Ceftria-xone	Cipro-floxacin	Genta-mycin	Imipenam
S. aureus (n = 39)	S	39 100%	39 100%	39 100%	34 87.2%	31 79.5%	39 100%	28 71.8%	33 84.6%	39 100%
	I	0	0	0	0	0	0	0	0	0
	R	0	0	0	5 12.8%	8 20.5%	0	11 28.2%	6 15.5%	0
S. epidermidis (n = 22)	S	22 100%	22 100%	16 72.7%	20 90.9%	22 100%	22 100%	22 100%	22 100%	22 100%
	I	0	0	0	0	0	0	0	0	0
	R	0	0	6 27.3%	2 9.1%	0	0	0	0	0
S. saprophyticus (n = 12)	S	12 100%	12 100%	12 100%	11 91.7%	12 100%	12 100%	12 100%	10 83.3%	12 100%
	I	0	0	0	1 8.3%	0	0	0	2 16.7%	0
	R	0	0	0	0	0	0	0	0	0
S. pyogenes (n = 10)	S	10 100%	10 100%	7 70%	10 100%	10 100%	10 100%	10 100%	10 100%	10 100%
	I	0	0	0	0	0	0	0	0	0
	R	0	0	3 30%	0	0	0	0	0	0

Keys: S = Susceptible or Sensitive; I = Intermediately Susceptible/Sensitive and R = Resistant

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Table 5b: *Antibiotic susceptibility and resistance profiles of Gram-positive bacteria isolated from diabetic foot ulcer patients visiting selected visiting hospitals in southwestern Nigeria*

Bacteria		Line-zolid	Netil-micin	Oflo-xacin	Oxa-cillin	Peni-cillin	Pipera-cilin	Sulpha-methazole	Trime-thoprim	Vanco-mycin
S. aureus (n = 39)	S	39 100%	39 100%	39 100%	34 87.2%	39 100%	39 100%	39 100%	39 100%	28 71.8%
	I	0	0	0	0	0	0	0	0	11 28.2%
	R	0	0	0	5	0	0	0	0	0
S. epidermidis (n = 22)	S	22 100%	22 100%	22 100%	22 100%	18 81.8%	22 100%	22 100%	22 100%	9 40.9%
	I	0	0	0	0	0	0	0	0	0
	R	0	0	0	0	4 18.2%	0	0	0	13 59.1%
S. saprophyticus (n = 12)	S	12 100%	12 100%	12 100%	12 100%	9 66.7%	10 83.3%	12 100%	12 100%	2 16.7%
	I	0	0	0	0	0	0	0	0	2 16.7%
	R	0	0	0	0	3 25%	2 16.7%	0	0	8 66.7%
S. pyogenes (n = 10)	S	10 100%	9 90%	10 100%	10 100%	7 70%	10 100%	10 100%	10 100%	8 80%
	I	0	0	0	0	0	0	0	0	2 20%
	R	0	1 10%	0	0	3 30%	0	0	0	0

Keys: S = Susceptible or Sensitive; I = Intermediately Susceptible/Sensitive and R = Resistant

Table 6: *CLSI interpretive performance standard for antimicrobial disk susceptibility testing [18]*

Antibiotic	Conc. (μg)	S	I	R
Amikacin	30	≥ 17	15–16	≤ 14
Amoxicillin	30	≥ 17	-	≤ 16
Ampicillin	10	≥ 17	-	≤ 16
Ceftazidime	30	≥ 21	18-20	≤ 17
Cefazolin	30	≥ 15	-	≤ 14
Ceftriaxone	30	≥ 23	20–22	≤ 19
Chloramphenicol	30	≥ 18	13-17	≤ 12
Ciprofloxacin	5	≥ 21	16–20	≤ 15
Clindamycin	2	≥ 21	15-20	≤ 14
Gentamicin	10	≥ 15	13–14	≤ 12
Imipenem	10	≥ 23	20-22	≤ 19
Linezolid	30	≥ 21	-	≤ 20
Methicillin	5	≥ 19	16-18	≤ 15
Netilmicin	30	≥ 15	13-14	≤ 12
Ofloxacin	5	≥ 16	13–15	≤ 12
Oxacillin	2	≥ 22	-	≤ 21
Penicillin	10	≥ 15	-	≤14
Piperacilin	100	≥ 21	18-20	≤ 17
Sulfamethoxazole	25	≥ 30	26-29	≤ 25
Trimethoprim	5	≥ 16	11-15	≤ 10
Vancomycin	30	≥ 17	15–16	≤ 14

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Figure 4 showed the percentage susceptibility and resistance of Gram-negative bacteria isolated from diabetic foot ulcers to antibiotics. A high percentage (76.99%) of Gram-negative bacteria isolated was susceptible to the conventional antibiotics under study while 22.85% was resistant and a low percentage (0.15%) showed intermediate susceptibility. The percentage susceptibility and resistance of Gram-positive bacteria isolated from diabetic foot ulcers to antibiotics was shown in Figure 5. Gram-positive bacteria showed 93.75% susceptibility, 1.21% intermediate susceptibility and 5.01% resistance to the conventional antibiotics investigated in this study

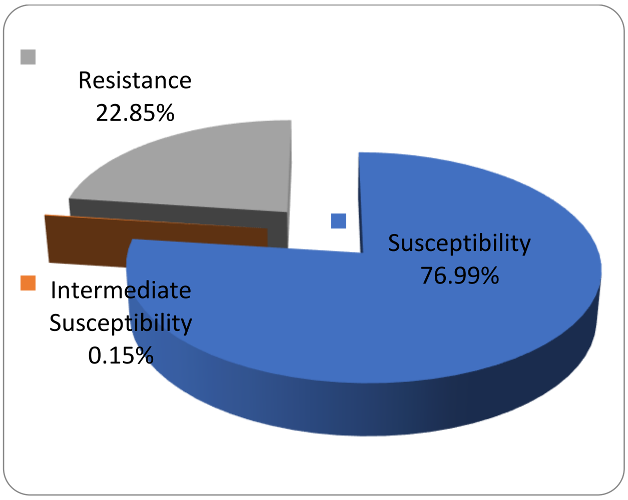

Figure 4: *Percentage susceptibility and resistance of Gram-negative bacteria isolated from diabetic foot ulcers to antibiotics*

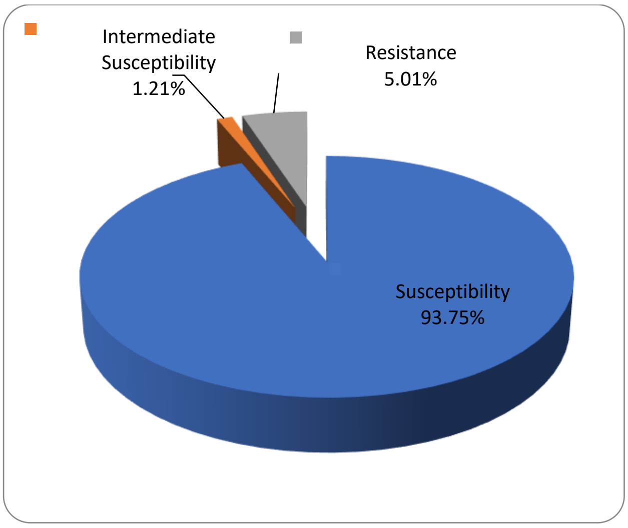

Figure 5: *Percentage susceptibility and resistance of Gram-positive bacteria isolated from diabetic foot ulcers to antibiotics*

Staphylococcus aureus had the highest percentage occurrence, followed by *P. aeruginosa* among in-patients and out-patients. Among the in-patients, *Acinetobacter baumannii* had the lowest occurrence while *M. morganii* had the lowest among the out-patients. *S. aureus* remained the most predominant while *A. baumannii* had the least. This agrees with the results of Perim *et al.* [19], Ayesha [20] and Pradeep *et al.* [21]. This result deviated from the reports of Banashankari *et al.* [22] and Daniel *et al.* [23] who reported *E. coli*, next to staphylococci, as the predominant bacteria in foot ulcer. The authors reported some of the bacteria encountered in this study but in different frequency. There were a greater number of Gram-negative bacteria than Gram-positive bacteria associated with diabetic foot ulcers and more than one potential pathogen was recovered from the samples analyzed in this study which is in agreement with some other studies [12], [24], [25].

The antibiotic resistance profiles of *S. aureus* strains in this study were similar to the observations made by Joseph *et al.* [25] and Oates *et al.* [26] in their study on diabetic foot infection. It, however, differs from the report of Daniel *et al.* [23] who reported 100% resistance of *S. aureus* isolates to vancomycin. Vancomycin resistance in *Staphylococcus aureus* among diabetic patients with foot lesion was also reported by Oates *et al.* [26]. However, different susceptibility patterns to antibiotics have been shown by other studies and, mostly, vancomycin, amikacin and linezolid have shown good activity against the strains [27] - [30], which is in agreement with findings of the present study.

The challenge of antibiotic resistance is a major public health concern, due to its global dimension and alarming magnitude, although the epidemiology of resistance can exhibit remarkable geographical variability and rapid temporal evolution. The understanding of bacteria associated with diabetic foot infections (DFI) and their antibiotic susceptibility profiles is essential for appropriate treatment and infection eradication. The empirical initiation of antibiotic therapy in patients with serious infections is necessary to prevent systemic invasion by infecting bacteria while awaiting microbiology laboratory results [31].

Clinicians should consider the results of bacterial culture and susceptibility testing in the light of the clinical outcome of the infection for the empirical therapy regimen. Knowledge of the characteristics of infection, i.e., the type of bacteria commonly found and the clinical evidence of infections, can help in choosing an appropriate antibiotic, even if the culture reports are not available at the time of initiation of antibiotic therapy [32] - [33].

The 22.85% resistance exhibited by the bacteria associated with diabetic foot infection in this study is of medical relevance due to the possibility of transmitting those resistant genes to other bacteria sharing the same ecological niche, and thus, impairing the implementation of successful antibiotic therapy [34]. Antimicrobial resistance could be transmitted to the human population, hospitalized patients, and the hospital environment through other sources including animals, plant-based foods, fish, poultry, and other industries in which antibiotics are used for different purposes and may lead to emerging resistant strains of bacteria [35] - [37].

The high antibiotic sensitivity profiles recorded in this study differ from many other studies [38] -[44], where bacterial strains encountered exhibited high resistance to most of the antibiotics investigated. This apparently revealed the variations in antibiotic sensitivity pattern of bacteria isolates based on geographical area. Bacterial strains, encountered in one geograpghical area or country are, in most cases, genetically different from others.

4 Conclusions

This study revealed that there is no definite aetiologic bacterial agent for diabetic foot infections and many of the associated bacteria are sensitive to certain antibiotics. Many bacteria that had been previously reported by many authors to be resistant to certain antibiotics were found to be susceptible to the antibiotics used in this study. Thus, many multi-drug resistant bacteria which could complicate the management of diabetic foot infections could be treated by the reported antibiotics. The study showed that many potential pathogens are associated with foot ulcers and which could pose serious health havoc if prompt therapeutic intervention is not made. This finding could assist clinicians to develop antibiotic therapy policy for the early treatment of diabetic foot infections in southwestern Nigeria.

5 Declarations

5.1 Acknowledgements

Appreciations to the Research Ethics Committees of UCH, Ibadan and OOUTH, Sagamu, Nigeria for the permissions to obtain samples from patients visiting the teaching hospitals.

5.2 Study Limitations

There is paucity of information on factors responsible for prevalence of diabetic foot infection in this study. Further research is needed to investigate the patients' attitude with respect to knowledge and practice of chronic wound care. In-depth information gained from current reports will be useful in developing risk-assessment model for a larger prospective cohort study. Metagenomic analysis on whole genome sequences of connected tissues is necessary to determine the microbial diversity associated with diabetic foot infections.

5.3 Ethical Approval

Ethical approval was given by Research Ethics Committees of UCH, Ibadan and OOUTH, Sagamu to promote this research.

5.4 Informed Consent

Informed consent to carry out research and disseminate findings were obtained from participants.

5.5 Funding Source

None

5.6 Competing Interests

Author declared, no potential conflict of interest exists.

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